Copper: an antioxidant nutrient for cardiovascular health

Kenneth G.D. Allen and Leslie M. Klevay*

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, Colorado and *United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota, USA

Dietary copper often is low in the Western diet; low intakes may affect all stages of atherosclerosis adversely. Impaired oxidative defense in copper deficiency contributes to hypercholesterolemia, hypertension, and impaired prostaglandin metabolism. Free copper ion does not exist *in vivo*; some in-vitro experiments are conducted with millions-fold excesses.

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Introduction

Approximately one-third of the elements in the periodic table have been related to the atherosclerotic process [1–3,4••]. One-third of these elements, in turn, produce relevant biological effects by enhancing or inhibiting copper [4••]. Copper will be emphasized here for the sake of both brevity and unity because of its importance to many (perhaps all) of the temporal stages of atherosclerosis (*vide infra*). Three major consequences of copper deficiency — hypercholesterolemia, hypertension, and thrombosis — are the result of impaired defense against oxidative damage.

Copper and lipid metabolism were linked 20 years ago when excessive zinc ingestion induced mild copper deficiency and hypercholesterolemia in rats [5,6,7••]. Hypercholesterolemia from copper deficiency without excess zinc [8] has been confirmed in many laboratories and several species and is generally accepted [9,10]. Since then, nearly 70 anatomical, chemical, and physiological similarities between animals deficient in copper and people with ischemic heart disease have been collected [11,12••] from hundreds of experiments published since 1928, when copper was shown to be an essential nutrient [13]. The first adverse effects of copper deficiency on the cardiovascular system were found little more than 10 years later [14,15]. Recently, men and women have been found to respond to diets low in copper, with potentially harmful changes in lipids [16,17], glucose tolerance [18], blood pressure [19], and electrocardiograms [16,20].

The western diet so closely associated with heart disease risk seems to be low in copper [11,21••]. Data from 10 dietary surveys were evaluated [11] and pooled [21••]. One-third of the chemically analysed diets contained less than 1 mg of copper/day and 61% contain less than 1.5 mg/day, which is the lower limit of the estimated safe and adequate intake in the USA [22].

The ready accessibility to diets low in copper, the numerous similarities between animals deficient in copper and people with ischemic heart disease, and the finding that people and animals respond similarly to diets low in copper have contributed to the copper deficiency theory of ischemic heart disease. This theory is consonant with much epidemiology and some iatrogenic maneuvers and experiments of nature [11,12••,23,24,25••].

Atherosclerosis begins very early in life [26,27]; its pathogenesis [28,29] involves an early accumulative stage when monocyte-derived macrophages accrue lipid to form foam cells. Foam-cell formation (lipid-laden macrophages) leads to development of the fatty streak, the earliest lesion of atherosclerosis and the progenitor of the mature occlusive lesion. These early inflammatory and later proliferative stages are part of a continuum of pathological change. The accumulation of lipid by macrophages involves oxidative damage to LDL by peroxidation of lipid components and modification of apolipoprotein B such that its interaction with the classical LDL receptor is impaired. Macrophages, however, express a scavenger receptor (the modified LDL receptor), which results in unregulated accumulation of modified LDL lipid components [30].

Copper as an antioxidant

The antioxidant role of copper resides in its catalytic function in copper-dependent superoxide dismutase [31]. Both cytosolic and extracellular copper-dependent superoxide dismutase activities have been characterized [32,33]. Enzyme activity can be decreased by diets low in copper [23,34].

Saari and Johnson [35,36] were the first to notice that antioxidants could decrease or prevent some of the

Abbreviation

HMG CoA—3-hydroxy-3-methylglutaryl coenzyme A.

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cardiovascular damage of copper deficiency. Exhaled (breath) ethane is increased in dietary copper deficiency, suggesting a generalized, or global, increase in lipid peroxidation [37]. Increases in aortic lipid peroxides in copper-deficient aortas have been reviewed [38•]. The oxidative changes in VLDL and LDL from copper-deficient rats have been demonstrated recently [39••].

This effect is most probably due to depression of both cytosolic and extracellular superoxide dismutase activity by reduced copper availability. Copper-marginal rats fed 2 μg copper/g diet, approximately 50% of the National Research Council recommended dietary copper concentration, have depressed aortic tissue copper-dependent superoxide dismutase activity [40]. Endothelial damage, assessed by scanning electron microscopy, in copper-marginal rats showed disruption of endothelial integrity and bulging of endothelial cells. With an additional challenge of 0.7% dietary cholesterol, copper-marginal rats exhibited drastically increased endothelial cell damage, with evidence of subendothelial lipid-laden macrophage accumulation. Part of this challenge may have been the result of the adverse effect of dietary cholesterol on copper metabolism [41,42]. Copper complexes have been found to be effective in preventing lipid peroxidation *in vivo* [43]. Superoxide dismutation is crucial in limiting oxyradical formation and in controlling lipid peroxidation [31].

Hypercholesterolemia in copper deficiency

In-vitro studies with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase purified from hepatic microsomes of rats have shown that glutathione is an effective regulator of enzyme activity over the normal hepatic glutathione concentration range [44,45]. The mechanism of in-vitro control of HMG CoA reductase activity involves glutathione-mediated reversible S-thiolation, a novel form of post-translational modification of enzyme activity. Thus, glutathione regulation is a competent mechanism for the regulation of HMG CoA reductase activity.

The hypercholesterolemia of copper deficiency is mediated, *inter alia*, by an increase in HMG CoA reductase activity [46,47]. The mechanism appears to involve reduced glutathione-mediated activation of HMG CoA reductase activity [48••]. Dietary copper deficiency causes increased hepatic and circulating concentrations of glutathione [49••] and also increased isolated incubated hepatocyte glutathione synthesis rates by two- to threefold [50••]. When copper-deficient rats are provided with L-buthionine sulfoximine, a specific inhibitor of glutathione synthesis, at amounts sufficient to normalize hepatic glutathione concentrations, both the hypercholesterolemia and increased HMG CoA reductase activity of copper deficiency are abolished [48••]. These results indicate that reversible S-thiolation of HMG CoA reductase activity, mediated by increases in glutathione, are responsible for the hypercholesterolemia of copper deficiency.

Hypertension in copper deficiency

Adult rats deficient in copper are hypertensive [51–53], with blood pressures that resemble those of spontaneously hypertensive rats [50••]. Altered interactions between vascular smooth muscle cells and endothelium-derived relaxing factor, identified as nitric oxide or a related nitroso compound, have been demonstrated in both large blood vessels and the microvasculature [54••,55••]. Both of these studies suggest that copper deficiency depresses vasodilation by alterations in endothelium-derived relaxing factor interaction with vascular smooth muscle. The mechanism for this effect could involve copper-dependent superoxide dismutase activity depression and hence decreased nitric oxide stability due to increased superoxide residence. Alternatively, alterations in guanylate cyclase activity and cyclic GMP could explain the altered responsiveness [55••] or depressed prostaglandin I_2 production [56••]. Guanylate cyclase purified from lung contains copper [57]. Some of these mechanisms have been reviewed [51,58•].

Platelets and prostaglandins in copper deficiency

During the latter stages of atherosclerosis, growth of the fatty streak leads to structural disruption of the endothelium allowing for platelet interaction [28]. Such platelet involvement causes a proliferative response involving platelet-derived growth factors. This proliferative phase leads to smooth muscle cell appearance in the intima, further lipid accumulation, and occlusion often associated with thrombosis. Both copper-deficient and copper-marginal diets have been shown to increase purified platelet thromboxane A_2 synthesis and to depress incubated aortic ring prostacyclin (prostaglandin I_2) production [56••,59••] in response to physiologically relevant agonist challenges. Both copper-marginal and copper-deficient diets depressed platelet and aortic copper-dependent superoxide dismutase activity; the increases in platelet thromboxane A_2 and decreases in aortic ring prostacyclin I_2 synthesis occurred with increased lipid peroxidation and hydroperoxide concentrations in both tissues [56••,59••]. These data are consistent with the known roles of lipid hydroperoxides in regulating prostaglandin H synthase and inhibiting prostacyclin synthase [60]. In both platelets and aortic ring incubations, Se-dependent glutathione peroxidase activity did not change in either copper-deficient or copper-marginal diets. Thus, dietary copper controls thromboxane A_2 : prostacyclin I_2 ratios and vascular homeostasis by copper-dependent superoxide dismutase-mediated changes in lipid peroxidation [56••] and lipid hydroperoxide concentrations [59••]. The importance of thromboxane A_2 in aggregation and vasoconstriction and of prostacyclin I_2 in anti-aggregation and vasodilation is well recognized in controlling platelet involvement in the proliferative stages of atherosclerosis [28,29]. Smooth muscle proliferation in copper-deficient arteries [11,61–63] was probably also mediated by these mechanisms. These changes in thromboxane and prostacyclin metabolism may explain partially why mice deficient in copper die with very large atrial thrombi [64] and may complement the impairment in the ability of these mice to dissolve blood clots [65••].

Some of these adverse affects [56••,59••,62] occur when dietary copper is similar to that in human diets [66], amounts that do not decrease standard clinical indices such as plasma copper [40,62] (superoxide dismutase, see above). No consistent change in plasma copper was found in human depletion experiments [16–20] on, for example, hypercholesterolemia (see above).

Apparent paradoxes solved

Oxidatively damaged LDL has been hypothesized to contribute to atherogenesis [30,67,68]. Superoxide ion may contribute to the process [30]; as noted above, copper-dependent superoxide dismutase is a probable defense. Oxidized lipoproteins isolated from atherosclerotic arteries have been found to be similar to lipoproteins oxidized *in vitro* with 10 μmol/l cupric sulfate. Heinecke *et al.* [69] were early students of this phenomenon. One should not infer from in-vitro oxidation of LDL by free copper (Cu^{2+}) that such a process occurs *in vivo* because free copper (Cu^{2+}) is unlikely to occur *in vivo* [70].

Copper is a member of the first transition series of elements [71]. In aqueous solution, ions of these elements form well defined aqua ions. Because these water molecules can be displaced completely by other ligands only with difficulty, one can infer that free copper (Cu^{2+}) never occurs in aqueous media. May *et al.* [72,73] have evaluated available data on the chemistry of copper complexes in blood plasma. Whereas total copper in blood plasma is approximately 1.8×10^{-5} mol/l, exchangeable copper is approximately 1 μmol/l. A large percentage of the latter is bound to albumin. They considered the usual concentration of 40 ligands (mostly amino acids) in plasma, appropriate data on formation constants, and some characteristics of protein binding. Computer simulation revealed that complexes with histidine and cystine predominate and that the average concentration of free copper (Cu^{2+}) was 10^{-18} mol/l, with a conservative upper limit of 10^{-11} mol/l. Mean free iron (Fe^{3+}) was 10^{-23} mol/l. Thus, micromolar concentrations of copper sulfate used in some in-vitro experiments are millions-fold higher than those physiological.

Journals devoted to inorganic or bioinorganic chemistry reveal both the power and the versatility of copper as a chemical reactant. One should not be surprised that copper sulfate will participate in chemical change when mixed with biochemicals *in vitro*. Biological, epidemiologic, and medical inferences from these observations must be based on the physicochemical reality of copper in aqueous solution. Sorenson [74•] and Gutteridge *et al.* [70] also have written on these concepts. The latter authors emphasize in particular the vanishingly low concentrations *in vivo* of copper bound to small molecules such as amino acids.

A high concentration of copper in serum is associated with high risk of death from cardiovascular disease [75] and acute myocardial infarction [76]. High serum ceruloplasmin (which correlates highly with serum copper) is suggested as a risk factor for myocardial infarction [77•]. In Northern Ireland, a positive relationship exists between high serum copper and the aggregation of classical risk factors [78•]. These observations, which may seem incongruous when juxtaposed with the copper deficiency theory, are not in conflict with the theory, however. High serum copper does not prove high copper nutriture; in fact, experiments with animals reveal that the opposite may be true [41,79••]. Measurement of copper in plasma or serum is insufficient for assessing nutritional status [80]. Reunanen *et al.* [77•] noted that it is 'unlikely that the high ceruloplasmin levels ... reflect high dietary intake or a high level of copper storage in the body'.

Other relevant observations

Cardiovascular mortality again has been found to be inversely related to water hardness [81•]. The relationship of this phenomenon to the copper deficiency theory has been reviewed; calcium and magnesium in hard water seem to improve copper use [82].

Singh *et al.* [83•] have reviewed the relationships of some chemical elements to coronary heart disease. Lysyl oxidase is a copper metalloenzyme essential for synthesis of normal elastin, collagen, and proteoglycans [23,24,84,85].

Hitomi-Ohmura *et al.* [86•] confirmed many observations in the past 2 decades [87] on the hypercholesterolemic affect of histidine and noted that HMG CoA reductase activity was increased. Extra dietary copper can abolish this hypercholesterolemic effect [88]. Histidine, a powerful chelating agent [89], increases the dietary requirement for copper and impairs its use. A similar phenomenon has been noted for the dietary combination of cholesterol with cholic acid [90], which has been used extensively to disturb lipid metabolism and induce atherosclerosis during the past 70 years.

Among Adventists in California, those who ate nuts regularly had fewer coronary disease events than those who ate nuts less frequently [91]. Higher intakes of copper may explain this beneficial effect because nuts generally are high in copper and because red meat (high consumption of which was associated with higher risk) is a poor source of copper [92••,93]. Similarly, higher intakes of copper [94•] may have contributed to the success of Ornish's lifestyle program [95].

Cholesterol feeding lowers liver copper in rabbits [41] and rats [42]. Vlad *et al.* [42] also found a decrease in aortic copper, which, along with the aortic damage, could be abolished by large doses of oral copper sulfate. Davidson *et al.* [96] are notable in their integration of the anatomy, chemistry, and physiology of copper deficiency. Some adverse effects, such as the prolonged QT interval in electrocardiograms, can be reversed on copper repletion.

Conclusion

Ischemic heart disease is the culmination of a slow, yet relentless, process that may take 50 years. Putative agents influencing this process should have harmful effects at all stages including the final event and be widely, but not uniformly, spread through the environment. Diets low in copper are readily accessible to populations with high prevalence of ischemic heart disease and thus satisfy these criteria.

Production of healthy connective tissue is dependent on a copper metalloenzyme, lysyl oxidase. Among the major risk factors altered unfavorably by copper deficiency, hypercholesterolemia is induced by excessive production of glutathione in liver, which activates HMG CoA reductase to increase plasma cholesterol. Insufficient activity of the copper metalloenzyme superoxide dismutase permits the oxidation of LDL, which then enters arterial cells more easily. Thromboxane and prostacyclin metabolism are affected adversely by increased arterial peroxide tone from impaired oxidative defense leading to both greater vascular tone and thrombogenesis. Clot lysis also is impaired. Hypertension exacerbates these effects. Dietary cholesterol exacerbates these effects by interfering with copper use. Free copper (Cu^{2+}) is virtually non-existent *in vivo* and does not affect these mechanisms. Dietary copper is an antioxidant nutrient essential for cardiovascular health.

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Kenneth G.D. Allen, Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523, USA.

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